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DOSE RESPONSE OF ELASTASE-INDUCED EMPHYSEMA IN HAMSTERS

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16. ABSTRACT

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Dose Response of Elastase-induced Emphysema in Hamsters[1-4]

JAMES A. RAUB, ROBERT R. MERCER, FREDERICK J. MILLER, JUDITH A. GRAHAM, and JOHN J. O'NEIL

SUMMARY Elastase-induced emhysema in hamsters was studied using pulmonary function tests in an effort to develop techniques for determining the effects of air pollutants on the progression of this disease. Single intratracheal injections of 6, 12, or 24 units of porcine pancreatic elastase produced dose-related changes in pulmonary function after 4 wk when compared with sham-injected control animals. Boyle's law end-expiratory volume and residual volume, measured by gas dilution, increased ($p < 0.05$) at 12 and at 24 units, respectively, whereas vital capacity, determined plethysmographically, and total lung capacity were increased ($p < 0.05$) at all 3 elastase doses. Respiratory system compliance, calculated by a nonlinear least squares regression fit of the deflation pressure-volume curve, increased ($p < 0.05$) at 24 units only. The multiple-breath nitrogen washout slope (N_2 slope) and the single-breath diffusing capacity for carbon monoxide (D_{LCO}) decreased ($p < 0.05$) at all 3 doses of elastase. Both histologic and physiologic evaluation showed dose-related pulmonary impairment. It appears, therefore, that as little as 6 units of elastase produces mild emphysema in hamsters, which is detectable by pulmonary function testing. Of these tests, the D_{LCO} and N_2 slope were the most effective in detecting the degree of impairment.

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Introduction

The elastase-induced emphysema model in hamsters has been used in a number of studies (1-8). However, different investigators have used varying amounts and purities of porcine pancreatic elastase and in many cases the elastase activity was not reported. In some of the elastase preparations, contamination with trypsin and chymotrypsin can be as high as 11% by weight (9). Snider and coworkers (4-6), Karlinsky and Snider (7), and Fedullo (8) used an elastase dose of 0.2 mg/100 g body weight, which produced a severe lesion resembling human panlobular emphysema (2, 7). Kaplan and associates (1) and Kuhn and Tavassoli (3) used 25 units of elastase to induce a lesion with similar characteristics. The present study investigated changes in the pulmonary function of hamsters treated with a dose range of elastase from 6 to 24 units. The goal was to establish a mild lesion that was detectable by the methods currently used for the evaluation of pulmonary function. It may be possible to use this disease model to study the interaction between air pollutants and experimental pulmonary emphysema. However, the model should be characterized by a well-defined but mild emphysematous lesion so that the study is not dominated by the disease.

Methods

Animals. Male golden hamsters weighing 98.3 ± 0.8 SEM g were divided into 5 groups (table 1). The animals were lightly anesthetized with halothane (Fluothane®) vaporized in air (Fluotec III, Cyprane, Inc., Tonawanda, NY) and placed on a tilted board (60°). A 22-gauge intubation needle was inserted into the trachea with the tip located midway between the larynx and the carina. The hamsters received either no treatment or a single (0.5 ml) instillation of buffer or 117.7 U/mg chromatographically purified porcine pancreatic elastase (Elastin Products Co., St. Louis, MO), which was assayed for activity by a minor adaptation of published methods (10). After treatment, the animals were returned to the animal facility for a 4-wk recovery period. Food and water were provided *ad libitum*.

Pulmonary function. The animals were anesthetized with sodium pentobarbital (Nembutal®), 50 mg/kg, given intraperitoneally. A tracheostomy was performed and a 14-gauge luer stub adapter (Becton-Dickinson, Rutherford, NJ) was tied into the airway. The hamsters were placed in a whole-body pressure plethysmograph connected to a large heat sink for isothermal conditioning, which produced a flat frequency response to 50 Hz. The tracheal adapter was connected to a bias flow through a small dead space valving system. Lung volume was obtained from changes in plethysmographic pressure measured by a differential pressure transducer (Model MP45; Validyne, Northridge, CA) referenced to a chamber equal in volume to the heat sink and connected to atmospheric pressure through a high impedance pathway. Airway pressure was measured by a differential pressure transducer (Model MP45; Validyne) referenced to the plethysmograph providing the pressure difference across the total respiratory system.

After a short equilibration period of resting breathing, the airway was occluded at end-expiration, and end-expiratory volume was calculated using Boyle's Law by recording airway pressure and plethysmographic pressure as the animal attempted to inspire against the closed airway. The animals were hyperventilated on a small animal respirator (Model 5255; Ealing Corp., Natick, MA) to increase minute ventilation and establish a uniform volume history. A pressure-volume curve was obtained during apnea by inflating and deflating the lungs with a syringe pump at 0.75 to 1.0 ml/s. Vital capacity was measured between a pressure difference of -15 cm H_2O and $+30$ cm H_2O. Respiratory system compliance was calculated by a nonlinear least squares regression fit of the deflation pressure-volume curve.

Multiple-breath nitrogen washout curves were obtained by ventilating the animals at a fixed volume and frequency with 100% oxygen while recording the breath-to-

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[2] This report has been reviewed by the Health Effects Research Laboratory, U. S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

[3] Presented in part at the Annual Meeting of the American Thoracic Society, May 1981.

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TABLE 1

GRAVIMETRIC DATA, LUNG WATER, AND HEMATOCRIT IN ELASTASE-TREATED HAMSTERS*

Group†	N	Body Weight (g)		Lung Weight (g)		Lung H_2O (%)	Hematocrit (%)
		Initial	Final	Wet	Dry		
C	10	99.1 ± 1.7	124.1 ± 2.8	0.704 ± 0.014	0.180 ± 0.003	74.4 ± 0.5	52.9 ± 1.0
ØU	10	98.1 ± 1.3	121.3 ± 2.7	0.702 ± 0.016	0.178 ± 0.004	74.6 ± 0.4	50.8 ± 1.3
6U	15	99.3 ± 2.0	117.6 ± 2.5	0.700 ± 0.015	0.183 ± 0.005	73.9 ± 0.3	‡
12U	12	97.4 ± 1.5	115.9 ± 2.8	0.825 ± 0.022§	0.205 ± 0.004§	75.1 ± 0.5	54.6 ± 1.3
24U	12	96.6 ± 1.9	104.1 ± 3.8§	0.874 ± 0.034§	0.210 ± 0.008‖	76.0 ± 0.4‖	57.3 ± 1.4‖

* All data are reported as mean ± standard error of the mean.
† Control (C), sham-injected (0U), 6 units (6U), 12 units (12U), and 24 units (24U) of elastase.
‡ Missing values.
§ Significance at $p < 0.01$.
‖ Significance at $p < 0.05$.

breath airway nitrogen concentration (Nitralyzer, Model 505; Med Science, St. Louis, MO). The slope of the log end-tidal nitrogen concentration versus breath number (N_2 slope) was used to assess the distribution of ventilation. Single breath diffusing capacity for carbon monoxide (D_{LCO}) and residual volume were measured by gas dilution techniques previously described from this laboratory (11).

After completion of the pulmonary function measurements, a sample of blood was taken for hematocrit determination. The animals were then exsanguinated and the lungs were removed. The heart and mediastinal tissue were dissected free and the lung weights were recorded before (LW_{wet}) and after (LW_{dry}) 24 h of air drying at 30 cm H_2O pressure and 105° C. Wet-to-dry ratios were calculated as per cent lung water ($LW_{wet} - LW_{dry}/LW_{wet} \times 100$).

Morphologic evaluation. Fifteen hamsters were used to evaluate the histopathologic changes induced by the porcine pancreatic elastase and confirm the presence of emphysema. Three animals were studied in each of the five previously defined groups. Each animal was lightly anesthetized with Nembutal and a tracheostomy was performed. A 14-gauge luer stub adapter (Becton-Dickinson) was tied into the trachea. The animal was exsanguinated by cutting the abdominal aorta, and the lungs were collapsed by bilateral puncture of the diaphragm. The lungs were expanded by gravity using 2% cacodylate-buffered glutaraldehyde at 30 cm H_2O pressure through a low-resistance fixation system attached to the tracheal cannula (12). After airway perfusion, the trachea was ligated and the lungs were excised and stored in a solution of the fixative. Sagittal sections of the left lobe and right diaphragmatic lobe were processed and stained with hematoxylin-eosin.

Statistical analysis. The effects of elastase instillation were evaluated statistically by comparing the weight-adjusted means of each pulmonary function measurement obtained using a one-way analysis of covariance (ANCOVA). When the F values from the ANCOVA were significant ($p < 0.05$), comparisons of each elastase dose group with the control mean were made using Dunnett's test (13).

Results

The effects of elastase treatment on body weight and lung weight after the 4-wk recovery period are shown in table 1. Although body weights were matched prior to treatment, there was a tendency for the hamsters with elastase-induced emphysema to gain less weight than either the nontreated (C) or sham-injected (ØU) animals. This effect was directly related to the dose received, but the differences were only significant at the highest dose (24U) when compared with ØU. There was a tendency for both wet and dry lung weights to increase over the same dose range of elastase. The differences were significant for 12U and 24U. There were also significant increases in per cent lung H_2O and hematocrit at 24U (table 1).

Pulmonary function. The trend of decreasing animal weight with increasing elastase dose was accounted for by adjusting the pulmonary function values for body weight using a one-way analysis of covariance. Because there were no significant differences between C and ØU for any of the pulmonary function measurements, all exposed groups were compared with the sham-injected animals. A dose-related increase in end-expiratory volume and residual volume, which was significant at 12U and 24U of elastase, and an increase in total lung capacity, which was significant at all 3 doses of elastase, are shown in figure 1A. There was a dose-related increase in compliance of the total respiratory system, which was significantly different from that in the control animals at the highest (24U) elastase dose (figure 1B). Significant decreases were measured in both N_2 slope (figure 1C) and D_{LCO} (figure 1D) at all 3 elastase doses.

Because an effect was observed at all 3 elastase doses, 3 units (3U) of elastase were evaluated in 16 additional hamsters and compared with an equal number of sham control animals (table 2). There were no statistical differences between any of the measurements.

Morphologic evaluation. Histopathologic lesions resembling emphysema were present in all hamsters given elastase. The severity of the lesion was dose dependent, ranging from mild focal emphysema in the alveolar ducts of hamsters given 6U of elastase to greater involvement of the terminal portion of the respiratory unit, the alveolar duct, the alveolar sac, and associated alveoli in hamsters given 12U and 24U of elastase. Intra-alveolar pigmented macrophages were also present, indicating alveolar wall rupture with subsequent alveolar hemorrhage. Remnants of the ruptured alveolar walls were present around the areas of emphysema. In the 2 high dose groups, alveolar wall thickening, focal bronchiolar, and Type II epithelial hyperplasia, and the presence of mononuclear cells and neutrophils suggested an elastase-induced alveolitis in the terminal portion of the respiratory unit.

Discussion

The results of the current study indicate that a dose response to elastase is measurable by conventional small mammal pulmonary function techniques. The question arises, however, that once a maximal effect is obtained, can additional damage induced by exposure to chemicals be measured? The pathologic and functional damage produced at the highest elastase doses (12U and 24U) is overwhelming. The effect of elastase on body weight was significant at 24U and a trend was indicated at 12U. Lung weights and volumes, lung compliance, gas diffusion, and the distribution of ventilation were all maximally affected by the higher elastase doses. A desirable model system of emphysema for use in environmental toxicologic studies should not exhibit impaired function to the extent that any additional damage would be obscured. This is particularly true if there is a treatment-related effect on lung and body growth and development.

The measurements of lung function changes induced by elastase treatment are similar to those reported by other

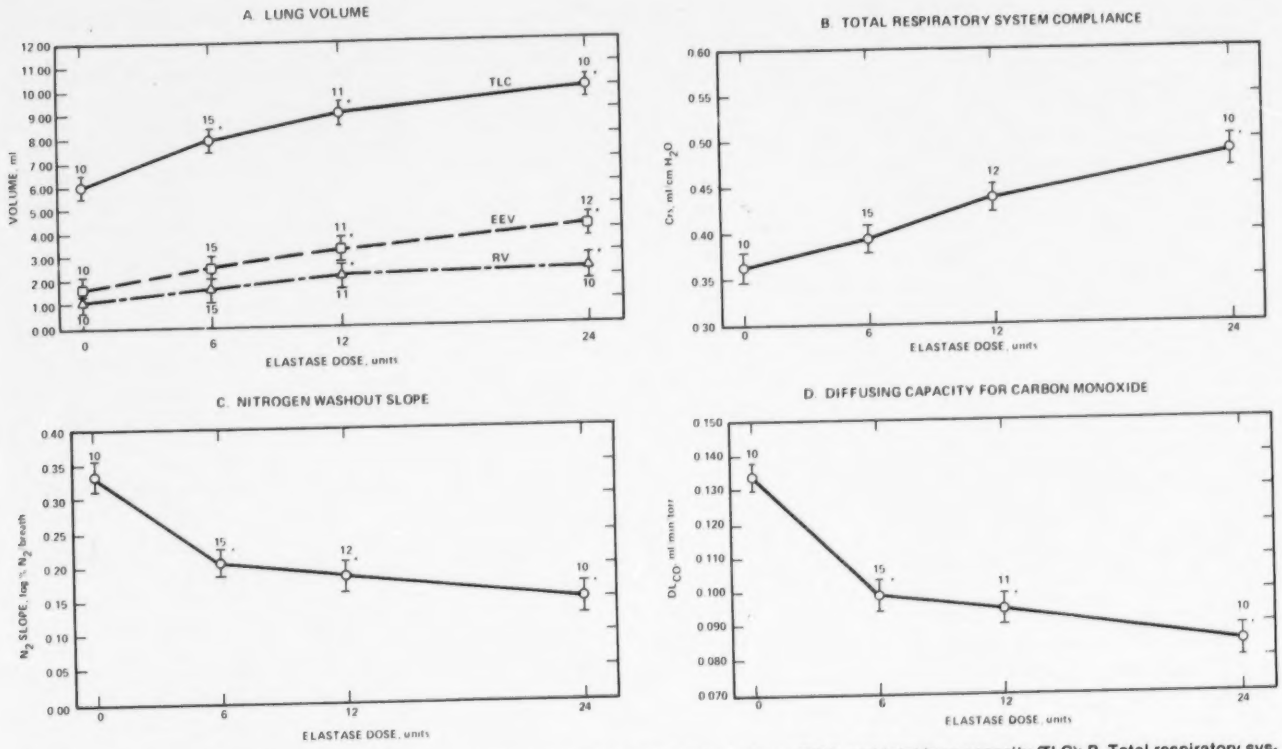

Fig. 1. Elastase dose response for A. Lung volume: residual volume (RV), end-expiratory volume (EEV), and total lung capacity (TLC); B. Total respiratory system compliance (Crs); C. Nitrogen washout slope (N_2 slope), as an index of the distribution of ventilation; D. Diffusing capacity of carbon monoxide (D_{LCO}). All values are the weight adjusted means obtained using a one-way analysis of covariance ± standard error of the mean. The number of observations for each dose is indicated. An asterisk denotes significance at $p < 0.05$.

investigators. Increases in lung volume and compliance have been shown by Snider and coworkers (4) and by Snider and Sherter (5) as well as an increase in the mean linear intercept and a decreased internal surface area that were not evaluated in the current study. The same investigators also reported decreases in body weight and increases in lung weight in the animals receiving elastase. Only one previous study (14) investigated dose response to elastase using lung volume and carbon monoxide diffusing capacity as indexes of lung function. Goldstein and coworkers (14) found that the end-expiratory volume measured by neon dilution increased from 1.23 ml to 4.4 ml and total lung capacity increased from 5.49 to 11.33 ml when control hamsters were compared with those receiving an elastase dose of 0.3 mg/100 g body weight. The results were strikingly similar to the high dose response in the current study where 0.2 mg/100 g body weight (24U) was used. They also reported that the rebreathing D_{LCO} did not correlate as well with the pathologic destruction as the calculated diffusion constant. This was attributed to an increase in lung volume that compensated for the alveolar wall destruction induced by elastase. However, the single-breath D_{LCO} appears to be a good indicator of diffusing surface loss based on the results in the current model system because there was a significant decrease in D_{LCO} at all three elastase doses. The severity of emphysema can also be evaluated by a change in the distribution of ventilation as shown by the N_2 washout slope, which decreased at each elastase dose, whereas the end-expiratory volume was increased at only the two highest doses of elastase. Therefore, only a portion of the effect on N_2 slope was probably due to increased end-expiratory volume.

The severity of the pathologic lesion

TABLE 2

PULMONARY FUNCTION MEASUREMENTS IN HAMSTERS RECEIVING A SINGLE INTRATRACHEAL INSTILLATION OF BUFFER OR 3 UNITS OF PORCINE PANCREATIC ELASTASE

	Buffer (n = 16)	Elastase (n = 16)
BWT, g	123.4 ± 2.3*	128.5 ± 1.7
LW_{wet}, g	0.642 ± 0.015	0.703 ± 0.014
LW_{dry}, g	0.160 ± 0.006	0.171 ± 0.004
Lung H_2O, %	75.2 ± 0.6	75.7 ± 0.3
RV, ml	0.65 ± 0.05	0.66 ± 0.05
EEV, ml	1.75 ± 0.04	1.79 ± 0.06
VC, ml	5.17 ± 0.10	5.44 ± 0.20
TLC, ml	5.83 ± 0.12	6.11 ± 0.21
Crs, ml/cm H_2O	0.407 ± 0.136	0.445 ± 0.023
N_2 slope, log % N_2/breath	0.391 ± 0.009	0.367 ± 0.013
D_{LCO}, ml/min/mmHg	0.123 ± 0.009	0.113 ± 0.003

Definition of abbreviations: BWT = body weight; LW = lung weight; RV = residual volume; EEV = end-expiratory volume; VC = vital capacity; TLC = total lung capacity; Crs = compliance of the total respiratory system; D_{LCO} = carbon monoxide diffusing capacity.

* All data reported as mean ± standard error of the mean.

that developed as a result of elastase treatment was also dose dependent. This is similar to the results obtained by Hayes and coworkers (2). The bronchial and parenchymal extent of this damage correlates with the functional changes reported in the current study.

There are only a few studies (15, 16) showing elastase-related activity at low doses (4 units) where morphologic and pathologic evidence of emphysema has been detected. Ip and coworkers (17) reported a maximal elastolytic change produced 24 h after the intratracheal instillation of 5 units of elastase. However, no functional lung measurements were reported in these studies and, in the current study, 3 units of elastase did not produce any significant change in lung function.

In conclusion, the results of pulmonary function measurements in hamsters over a range of elastase doses indicates that 6 units of porcine pancreatic elastase produces a mild but significant lesion indicative of emphysema, both pathologically and functionally, but without the gross pathologic involvement shown at higher doses used by others. This dose imparts a degree of pulmonary function impairment that allows study at various stages in the progression of this disease and under differing environmental conditions.

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